

ORIGINAL RESEARCH ARTICLE

Co-testing in cervical screening among 40- to 42-year-old women is unreasonable

Christer Borgfeldt¹ | Anneli Leksell² | Ola Forslund³ 

¹Department of Obstetrics and Gynecology, Skåne University Hospital, Lund University, Lund, Sweden

²Department of Clinical Genetics and Pathology, Laboratory Medicine Region Skåne, Lund, Sweden

³Department of Medical Microbiology, Laboratory Medicine Region Skåne, Lund University, Lund, Sweden

Correspondence

Ola Forslund, Department of Medical Microbiology, Laboratory Medicine Region Skåne, 221 85 Lund, Sweden.
Email: ola.forslund@med.lu.se

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Abstract

Introduction: The screening program for cervical cancer in Sweden recommends the use of primary human papillomavirus (HPV) screening for women aged ≥ 30 to 65 years. Co-testing with both HPV analysis and cytology is recommended at the first screening after the age of 40 years. To fulfil co-testing, all screened women aged 40–42 years within the region of Skåne were co-tested. The aim of the audit was to investigate the proportion of severe dysplasia as diagnosed by cytology and histological follow-up among women with Aptima HPV-negative tests. We also calculated the cost of adding the cytology to the HPV primary screening program.

Material and Methods: The local cytology registry was used to identify women aged 40–42 years who attended screening and were co-tested during the 4 years from January 2017 to December 2020. The Aptima HPV messenger RNA assay detects 14 HPV types. For Aptima HPV-negative women with high-grade cytology or histological high-grade squamous intraepithelial lesions (HSILs), we performed extended HPV typing for 40 HPV types with polymerase chain reaction using modified GP5+/6+ primers followed by a Luminex assay. To estimate the added cost of using cytology to identify each histologically confirmed cervical HSIL case among Aptima HPV-negative women, we used the current cost of €21.2 per cytology evaluation at our laboratory.

Results: Of 19 599 women, 5.8% (1137/19 599) had abnormal cytology. Among Aptima HPV-negative women, 0.11‰ (2/18 132) had histologically confirmed HSIL. One of the women was infected with HPV18 and the other with HPV73 at the diagnosis of HSIL. The calculated cost to find one HSIL, by adding cytology to HPV-negative cases, was approximately €200 000.

Conclusions: The clinical benefit of a single cytology co-test added to an HPV-based screening program in women aged 40–42 years appears doubtful and economically unreasonable.

Abbreviations: AGC, atypical glandular cells; ASC-H, atypical squamous cells of undetermined significance cannot exclude HSIL; ASCUS, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; MGP, modified general primers; RLU, relative light units.

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KEYWORDS

cervical screening, co-testing, cytology, HPV, organized screening program

1 | INTRODUCTION

Several industrialized countries have successfully implemented cervical cancer screening programs for the detection of cervical precancers in cytology samples.¹ Human papillomavirus (HPV) can cause cervical cancer,² and this discovery led to the use of primary HPV screening because of its higher sensitivity but lower specificity in the detection of high-grade cervical intraepithelial neoplasia (CIN2+) compared with cytology.³

True HPV-negative cervical cancers are rare,⁴ but a small proportion of HPV-positive cancers and pre-cancers might be overlooked by primary HPV screening assays restricted to detection of the 14 established high-risk HPV types, such as the Cobas 4800 HPV Test (Roche Molecular Diagnostics), Real-Time High Risk HPV test (Abbott Molecular), BD Onclarity HPV assay (Becton, Dickinson and Company), and Aptima HPV Assay (Hologic, San Diego).^{5–8}

Co-testing with both primary HPV testing and cytology has been described as a tool to gain higher sensitivity for the detection of cervical high-grade squamous intraepithelial lesions (HSILs) compared with HPV testing alone.⁹ Interestingly, the screening strategy preferred by the American Cancer Society is primary HPV testing every 5 years, with co-testing and cytology alone acceptable where access to US Food and Drug Administration-approved primary HPV testing is not yet available.¹⁰ However, the screening program for cervical cancer in Sweden recommends the use of primary HPV screening for women aged ≥ 30 to 64 years. In addition, co-testing for both HPV and cytology is recommended at the first screening after the age of 40 years.¹¹ The Swedish National Cervical Cancer prevention working group decided that co-testing using both cytology and HPV analyses should be performed in women around the age of 40 years to ensure detection of all abnormal cytology samples, even for the few cases that can occur without an active HPV infection.¹¹ To fulfil co-testing, samples from all screened women within the region of Skåne, Sweden, aged 40–42 years are co-tested. The aim of the present audit was to investigate whether co-testing of primary HPV-negative screening samples might be useful to identify women with high-grade cervical lesions. We also evaluated and calculated the cost of adding the co-testing with cytology to the HPV primary screening program.

2 | MATERIAL AND METHODS

Cervical cell samples were collected by midwives in ThinPrep liquid-based transport devices (Hologic). Cytology and HPV testing were performed at Clinical Pathology, Lund, Sweden. This laboratory performs all cytology and HPV testing in the region of Skåne. Cytology diagnoses were set according to the Bethesda system¹² by

Key message

Primary human papillomavirus (HPV) screening was introduced in Sweden in 2017 and includes co-testing of women aged 40–42 years. Among Aptima HPV-negative women, 0.11% had histological high-grade squamous intraepithelial lesions, at a cytology cost of €200 000 per case. The clinical and economic benefit of cytology added to HPV-based screening appears unreasonable.

cytotechnologists with knowledge of the HPV test results. For the Aptima HPV analysis, 1 ml of the sample was automatically transferred (Tomcat, Hologic) to an Aptima specimen transfer tube (pre-filled with 2.9 ml buffered solution). The Aptima HPV assay (Hologic) was performed according to the manufacturer's instructions using the Panther platform (Hologic). The Aptima HPV messenger RNA assay detects 14 high-risk HPV types simultaneously (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68).¹³ We used the local cytology registry to identify screened women who were co-tested. Overall, 19 655 women aged 40–42 years were co-tested (Figure 1) in the 4 years from January 2017 to December 2020. In 2017 to January 2019, all HPV-negative women with atypical squamous cells of undetermined significance (ASCUS) or worse were referred for colposcopy. From February 2019 to December 2020, only HPV-negative women with cytology results indicating HSIL or worse (including atypical glandular cells [AGC] or adenocarcinoma in situ) were referred for colposcopy. For Aptima HPV-negative women with high-grade cytology or histological HSIL, we performed HPV typing with polymerase chain reaction using modified GP5+/6+ primers followed by Luminex assay for 40 mucosal HPV types (HPV type 6, 11, 16, 18, 26, 30, 31, 33, 35, 39, 40, 42, 43, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68 (a and b), 69, 70, 73, 74, 81, 82, 83, 85, 86, 87, 89, 90, 91 and 114).^{14,15}

To estimate the added cost of cytology and to identify each histologically confirmed cervical HSIL case among Aptima HPV-negative women, we used the current cost of €21.2 per cytology evaluation at our laboratory.

2.1 | Ethical approval

The study was approved by the regional ethics board in Lund (reference number DNR 2013/390 approved June 19, 2013; and amendment DNR 2018/466 approved May 18, 2018). The approval explicitly allows the investigation and publication of all screening data contained in the registry, including histopathological data and

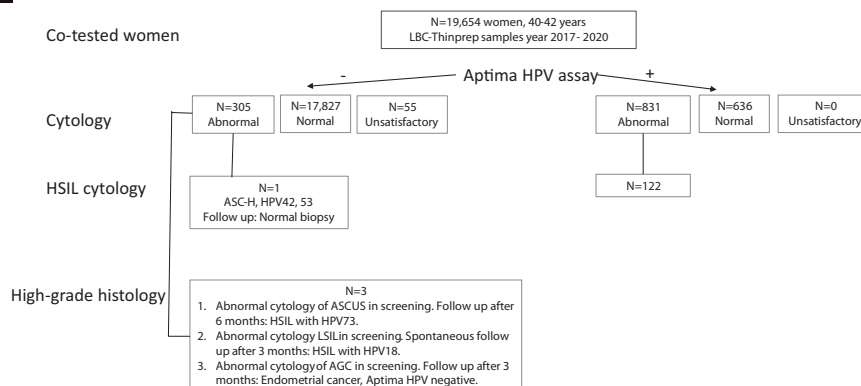


FIGURE 1 Overview of the 19 654 co-tested women aged 40–42 years within the region of Skåne, Sweden, during 2017–2020 and included in the study. The Aptima human papillomavirus (HPV) messenger RNA assay detects 14 HPV types. AGC, atypical glandular cells; ASC-H, atypical squamous cells of undetermined significance cannot exclude HSIL; ASCUS, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LBC, liquid-based cytology; LSIL, low-grade squamous intraepithelial lesion.

HPV results. Therefore, no further individual consent was required. Furthermore, all women who participate in the cervical screening program have the ability to opt out and delete their personal data from the registry at any time.

3 | RESULTS

We identified 19 654 screened women aged 40–42 years with co-testing results; 55 unsatisfactory cytology results were excluded, resulting in a total cohort of 19 599 co-tested women (Figure 1). Overall, 5.8% (1137/19 599) had abnormal cytology (Table 1). Among Aptima HPV-negative women, 0.11% (2/18 132) had histologically confirmed HSIL. The co-tested cytology from these women demonstrated ASCUS, whereas the others had low-grade squamous

intraepithelial lesions (LSILs). One of these women had HPV18 and the other had HPV73 (Table 2). We also observed one Aptima HPV-negative woman with abnormal high-grade cytology of atypical squamous cells of undetermined significance cannot exclude HSIL (ASC-H), with the presence of HPV42 and HPV53, who had benign histology in a biopsy (Table 2). Furthermore, the Aptima HPV-negative women also included one cytology of AGC where the woman had histologically confirmed endometrial cancer at follow-up (Table 2).

Overall, among Aptima HPV-positive women with abnormal cytology, 16.1% (134/831; 95% confidence interval 13.7–18.8) demonstrated high-grade cytology (Table 1).

The cost to find one cervical HSIL by adding cytology to the testing of women aged 40–42 years with HPV-negative results was calculated at approximately €200 000.

TABLE 1 Co-testing screening results of women aged 40–42 years within region of Skåne Sweden during 2017–2020

Cytology	Number	Aptima HPV positive (%)	Aptima HPV negative (%)
Normal	18 463	636 (3.4)	17 827 (96.6)
Abnormal cytology	1136	831 (73.1)	305 (26.9)
ASCUS	689	429 (62.3)	260 (37.7)
LSIL	307	268 (87.3)	39 (12.7)
HSIL	83	83 (100)	0 (0)
ASC-H	40	39 (97.5)	1 (2.5)
AGC	17	12 (70.6)	5 (29.4)
Total	19 599	1051 (5.4)	18 132 (92.5)

Aptima HPV messenger RNA assay detects 14 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68).

Abbreviations: AGC, atypical glandular cells; ASC-H, atypical squamous cells of undetermined significance cannot exclude HSIL; ASCUS, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

4 | DISCUSSION

Among co-tested screened women who were Aptima HPV negative, we observed a low rate (0.11%) of histologically confirmed high-grade cervical lesions. We also saw a single case of high-grade cytology with ASC-H, positive for HPV42 and HPV53, who had a benign biopsy at follow-up. Furthermore, one woman with Aptima HPV-negative AGC was diagnosed with endometrial cancer at the age of 41 years (corresponding to 0.005% of our co-tested cohort [1/19 599]). The value and purpose of co-testing for earlier detection of endometrial cancer within primary HPV screening among women aged 40–42 years appears dubious since the median age of women with endometrial cancer is around 60 years.¹⁶ Symptoms of post-menopausal bleeding or bleeding disturbances have been demonstrated as the most sensitive markers for endometrial cancer.¹⁷

Notably, two Aptima HPV-negative women with low-grade abnormal cytology (ASCUS and LSIL) had HSIL at follow-up after 3–6 months. Interestingly, for the woman with LSIL, a weak signal

TABLE 2 Abnormal cytology and histology follow-up among HPV-negative co-tested screening women aged 40–42 years within the region of Skåne, Sweden, during 2017–2020

Index cytology	Number	Attended follow-up (%)	Histology number	Histology
ASCUS	260	75 (29)	26	HSIL N = 1 (HPV73) ^a , LSIL N = 4, unclear atypia N = 1, normal N = 19, insufficient sample N = 1
LSIL	39	13 (33)	10	HSIL N = 1 (HPV18) ^b , LSIL N = 1, unclear atypia N = 1, normal N = 7
HSIL	0	—	—	—
ASC-H ^c	1	1 (100)	1	Normal N = 1
AGC	5	5 (100)	5	Endometrial cancer N = 1, ^d normal N = 4
Total	305	94 (31)	42	

Abbreviations: AGC, atypical glandular cells; ASC-H, atypical squamous cells of undetermined significance cannot exclude HSIL; ASCUS, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

^aOne woman had histologically confirmed HSIL with HPV73 at follow-up after 6 months.

^bOne woman had histologically confirmed HSIL with HPV18 at follow-up after 3 months.

^cOne woman had HPV42 and HPV53 in cytology and a normal biopsy after 3 months.

^dOne woman had histologically confirmed endometrial cancer after 2 months.

below cutoff (relative light units [RLU] 7598) was observed in the Aptima assay, but no HPV type was detectable. However, at follow-up 3 months later, the Aptima assay was positive (RLU 2088066, S/CO 17.15) with simultaneous identification of HPV18. It can be speculated that the index sample contained low copy numbers of HPV18 transcripts below the detection level for positivity with the Aptima assay and that the Aptima assay would have been positive if the screening sample had been collected some weeks later. The other woman with ASCUS had HPV73 at follow-up after 6 months. HPV73 is not detectable by the Aptima assay but has been suggested as an oncogenic HPV type.^{18,19} Recently, among Aptima HPV-negative liquid-based cytology samples, we observed HPV73 in 55.5% (5/9) of invasive cervical cancers and in 29.3% (22/75) of different grades of cervical diagnosis.²⁰ These findings warrant consideration of including HPV73 in primary HPV screening.

The strength of this study is the complete population-based screened cohort of women in a single region. One laboratory serves the whole region for all HPV, cytology, and pathology analyses. A limitation of the audit is that the criteria for follow-up among Aptima HPV-negative women changed during the study period. In the first 2 years of the study, all Aptima HPV-negative women with abnormal cytology were referred to colposcopy follow-up with biopsies if needed. During this period, 46% (59/129) attended follow-up. For the subsequent 2 years, referral was restricted to women with HSIL, AGC, and adenocarcinoma in cytology, so only 20% (35/176) of the corresponding women with abnormal cytology had follow-up. This may have led to the occasional missed histological high-grade lesion.

Within the region of Skåne, primary HPV screening is commenced at age 30 years, with co-testing only for women aged 40–42 years. The total cost of adding cytology co-testing for women aged 40–42 years with Aptima-negative samples was estimated at €393 297 during the 4 years of the study. Since only two cases

(0.11‰) of high-grade cervical lesions were confirmed among the 18 132 Aptima HPV-negative cases, the viability of co-testing using cytology in women aged 40–42 years in our screening cohort appears doubtful. In our study, the cost to find one HSIL with the addition of cytology to negative HPV analyses was approximately €200 000. However, Kaufman et al. reported that co-testing was more effective for the detection of cervical cancer.²¹ Despite this, Malinowski et al. concluded that including cytology in primary HPV screening provides little benefit in terms of sensitivity or diminution of risk compared with that of unaccompanied HPV testing.²² It is important that analyses in an organized screening program are reliable; however, in comparison with the coverage rate in the region of Skåne during the study period, where one in five women did not participate, cytology adds extremely little.

5 | CONCLUSION

Our audit indicates that the contribution of co-testing among Aptima HPV-negative women aged 40–42 years is limited and that the value of continued rounds of co-testing is questionable.

CONFLICT OF INTEREST

Ola Forslund has received a speech honorarium from Hologic, and his laboratory department (Laboratory Medicine, Region of Skåne) has ongoing contracts with Hologic. Christer Borgfeldt received a grant from Hologic Inc in 2018 to perform HPV studies. Anneli Leksell has no conflicts of interest.

AUTHOR CONTRIBUTIONS

CB and OF contributed to the study design and preparation of the manuscript. AL contributed to the obtaining registry data and quality check of results.

ORCID

 Ola Forslund  <https://orcid.org/0000-0001-6293-7286>

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